

Citrate synthase activity does not account for age-related differences in maximum aerobic performance in House Sparrows (*Passer domesticus*).

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ABSTRACT

We measured basal (BMR) and peak metabolic rates (PMR) in juvenile and adult House Sparrows. Juvenile birds had significantly higher BMR, but lower PMR than adult birds, despite having statistically indistinguishable body masses. We then evaluated the relation between PMR and masses of central and peripheral organs and found that pectoral muscle mass best correlated with PMR in both groups, accounting for about 35% of the variation in PMR. Because citrate synthase (CS) has such major importance in affecting the first committed step in the tricarboxylic acid cycle, we characterized CS activity levels in extracted muscles to see if this better explained age-related differences in peak aerobic performance. Surprisingly, juvenile sparrows had significantly higher CS activity levels than adults (197.8 vs. 179.0 $\mu\text{M g}^{-1} \text{min}^{-1}$, respectively). This higher enzyme activity in juveniles was completely offset by their significantly smaller proportion of flight musculature relative to body mass (17.7 % in adults vs. 15.3% in juveniles). Consequently, ontogenetic changes in relative sizes of organs best accounts for age-related differences in peak metabolic rate.

Key words: peak metabolic rate, citrate synthase, aerobic capacity, ontogeny, house sparrows

Introduction

From the time of the earliest hunters, humans have been fascinated by the large amount of variation in animal locomotor endurance. This interest is still prevalent and has provoked research focused on understanding the physiological and morphological factors affecting aerobic performance. These studies have led to diverging hypotheses as to which physiological/morphological components set the limits to a given species aerobic capability. Some have proposed that these limits are set by the capacity of peripheral effector organs (Peterson et al. 1990), by the capacity of central “supply” organs (Kirkwood 1983; Weiner 1992; Koteja 1996), or are associated with equal capacity of all components (symmorphosis; Weibel et al. 1991). One way to test these hypotheses is to compare proportionate sizes of peripheral and central organs among species that differ significantly in aerobic performance (e.g., Daan et al. 1991), or to do this among members of the same species showing a range of locomotor abilities. We previously used the latter approach to evaluate the morphological traits of juvenile and adult house sparrows (*Passer domesticus*) in relation to their basal and peak metabolic rates (BMR and PMR, respectively; Chappell et al. 1999). Because the sizes of both a central organ (heart) and a peripheral tissue (pectoral muscle) accounted significantly for variation in PMR, our results supported the symmorphosis model of aerobic capacity. Importantly, however, pectoral muscle mass best correlated with PMR and BMR among all tissues considered.

A tacit assumption of inferring organ capacity from mass alone is that the mass-specific capabilities of organs among species or of individuals within species are the same. Studies comparing the structure of mammalian locomotor muscles among ‘athletic’ versus ‘non-athletic’ species reveal the weakness of this assumption (Weibel et al. 2004). Their study showed that highly aerobic species had muscles with significantly greater mitochondrial and capillary densities than their less athletic counterparts. Although we did not characterise mitochondrial or capillary properties of sparrow locomotor muscles, we did save leg and flight muscle samples to subsequently examine citrate synthase (CS) activities. This enzyme catalyses the first step of the tricarboxylic acid cycle of mitochondrial respiration and is believed to be a surrogate of tissue aerobic capacity (Hochachka et al. 1988). If sparrows show substantial variation in pectoral muscle CS activity, then mass-specific aerobic capacity of these muscles should vary to the same extent. If this were so, we would expect measurements of total pectoral CS activity to correlate better with PMR than pectoral mass alone. We report here the effect of including CS activity in evaluating the relation between PMR, BMR and pectoral muscle mass in a subset of sparrows we studied previously (Chappell et al. 1999).

Material and Methods

Study Animals

We captured free-living house sparrows (*Passer domesticus*) residing in the Illawarra region of NSW between November and December. Birds at this time of year comprise adults and juveniles that are mostly 2-4 months old. We obtained 36 adults and 30 juvenile birds with near-equal gender distribution in each group for a study examining the relation between organ morphology and metabolic performance (Chappell et al. 1999). We have subsequently characterised citrate synthase activity in muscle samples from 32 of these adults (15 females, 17 males) and 28 of these juveniles (13 females, 15 males) and report these results in relation to their metabolic and morphological measurements. Because most methods involved in these comparisons are detailed in Chappell et al. (1999), only an overview is provided here.

Peak Metabolic Rate

On a given day, 4 birds were caught early morning and placed in a cage with *ad libitum* access to seed and water. About midday, each bird was placed in a motorised, revolving 5-litre drum that had clear sides and carpet lining the inner rim. A mass-flow controller supplied air at 5 l min⁻¹ and the gas content of inlet and outlet ports was measured with an oxygen analyser (Sable Systems FC-1). Birds were introduced into the chamber, allowed 2 min to settle, and then the motor was activated. The flight drum also contained ping-pong balls, which encouraged birds to maintain a series of rapid takeoffs and short-term flights. Measurements were terminated at the first indication of exercise fatigue by a bird. The metabolic data were transformed to 'instantaneous' values using methods described by Bartholomew et al. (2001). The highest 60-sec instantaneous oxygen consumption rate averaged over a continuous 60-sec interval was termed Peak Metabolic Rate (PMR). Birds were then returned to cages and allowed to feed and drink before measurement of basal metabolic rates (BMR).

Basal Metabolic Rate

Food was removed from cages at 1500 and each bird was removed at 1800 and then transferred to a respirometer fashioned from a 2-l paint can fitted with inlet and outlet ports, a perch, and a thermocouple to measure chamber temperature. The respirometers were housed in a temperature-controlled cabinet set to 32.5 °C, a thermoneutral temperature for this species (Hudson and Kimsey 1966), and were each supplied an airflow of 500 ml min⁻¹. Inlet and outlet oxygen concentrations were measured on a 2-channel oxygen analyser (Applied Electrochemistry S-3A) and recorded using an A-to-D converter (Data Electronics) connected to a Macintosh computer. Oxygen consumption measurements continued until the following morning, with the lowest continuous 10-min interval recorded over this period designated as BMR. Birds were then returned to cages and allowed to feed and drink for 2 hours before being killed for morphological characterization.

Morphological Measurements

Birds were weighed to the nearest 0.05 g prior to dissection. Organs were removed, trimmed of excess fat and connective tissue, rinsed with physiological saline, and blotted dry before being weighed to the nearest 0.1 mg. The contents of the heart and intestine were emptied before being rinsed, blotted, and weighed. Sampled organs included liver, lungs, gut (small and large intestines), gizzard, lungs, heart, kidneys, and reproductive organs. In addition, the entire right pectoral musculature and all leg muscles proximal to the tibiotarsus-tarsometatarsus joint were removed and weighed. All organs and muscle samples were then dried to constant mass at 60 °C and then reweighed. Samples of left pectoral muscles and left leg were also excised, weighed, and snap frozen in liquid nitrogen for subsequent enzyme analyses.

Citrate synthase measurements

Muscle samples were minced with fine scissors and a portion of these then weighed to the nearest 0.1 mg before being homogenised in 20 volumes of 100 mM potassium phosphate and 2 mM EDTA. Homogenisation was first performed using 3 10-sec pulses of an Ultra-Turrax T-25 tissue homogeniser while the sample was surrounded by ice, followed by 1-min of final homogenisation using ground-glass tissue grinders. Citrate synthase activity was measured using Dawson and Olson's (2003) modification of the method described by Srere (1969). This involved adding a 0.1 ml volume of the muscle homogenate to a final 1 ml volume comprising 100 mM Tris-HCl, 0.2 mM *s*-acetyl CoA, 0.1 mM DTNB (5,5'-dithiobis (2-nitrobenzoic acid)), 2.5 mM EDTA, 0.5 mM *cis*-oxaloacetate maintained at 25 °C. The reaction is initiated by addition of the oxaloacetate, with the rate of CoA-SH produced measured by rate of change in absorbance at 412 nm on a recording spectrophotometer (Beckman). All reagents and enzymes were purchased from the Sigma-Aldrich Chemical Co.

Statistical Analyses

Because most morphological and physiological features of animals are influenced by body mass, examination of gender and age effects on these variables were analysed using an ANCOVA general linear model, with gender and age as fixed factors and body mass as a covariate (SPSS). Correlation analyses were used to compare the amount of variance accounted by different regression models. Some post-hoc analyses employed T-tests, with multiple comparisons using Bonferroni correction factors to correct for accrual of Type I errors. The level of significance was $p < 0.05$ for all statistical comparisons. Unless stated otherwise, all values are reported as mean \pm one standard error.

Results and Discussion

Although house sparrows are known to be size dimorphic, these tendencies are greatly reduced in populations living in regions with limited annual variation in temperature (Murphy 1985). This may explain the near absence of sexual dimorphism in the traits we

measured in Illawarra birds. The only organs to differ between sexes were the slightly larger liver and gizzard in adult females compared to adult males (Chappell et al. 1999). By contrast, juvenile and adult sparrows differed significantly in a number of morphological characteristics. Compared to adults, juveniles displayed hypertrophied digestive organs (gut, gizzard, liver, and kidneys; Table 1). This enlargement most likely represents the retention of traits permitting very rapid growth as chicks by recently fledged juveniles (Klaassen and Drent 1991). In contrast to differences in digestive organ sizes, leg and pectoral muscles were significantly smaller in juveniles than in adults (Table 1; Fig. 1). Because these groups had statistically indistinguishable body masses, this implies that juveniles have potentially less locomotor capacity than adults. Apart from these morphological differences, there were significant age-related differences in both basal and peak metabolic rates (Table 1), with juveniles having higher BMR, yet lower PMR than their adult counterparts. It is worthwhile to see how the age-related differences in morphological features are associated with variation in aerobic metabolism.

One of the first studies to identify morphological influences on BMR concluded that interspecific variation was largely due to proportionate differences in the relative size of hearts and kidneys (Daan et al. 1990). This approach has been extended to evaluate intraspecific variation in BMR, but typically with the organs appearing to be responsible for this variation differing between species (e.g., Daan et al. 1990 versus Chappell et al. 1999) or at different times of year within species (Vezina and Williams 2003). It has been suggested that the lack of consistency in explaining variation in BMR is due to its reliance on the assumption that the organs being compared have the same metabolic intensity (Vezina and Williams 2005). Although we did not measure tissue-specific metabolic rate, the activity of citrate synthase (CS) is believed to be a surrogate of tissue aerobic capacity (Hochachka et al. 1988; Vezina and Williams 2005).

Table 1. Age effects on morphological and physiological variables of 32 adult and 28 juvenile house sparrows. Statistical comparisons for all variables except body mass are based on ANCOVA with body mass as a covariate. Body mass differences between age classes are based on ANOVA. P values of less than 0.05 identify statistically significant differences between adults and juveniles.

Variable, units	Adults	Juveniles	P adults vs. Juv.
Body Mass, g	23.10 ± 0.35	22.34 ± 0.40	0.15
Lungs, g	0.246 ± 0.009	0.251 ± 0.005	0.28
Heart, g	0.262 ± 0.007	0.241 ± 0.004	0.051
Gizzard, g	0.872 ± 0.028	1.054 ± 0.037	<0.0001
Liver, g	0.619 ± 0.018	0.786 ± 0.020	<0.0001
Gut, g	0.430 ± 0.017	0.659 ± 0.023	<0.0001
Kidneys	0.240 ± 0.006	0.284 ± 0.005	<0.0001
Pectoral muscles, g	2.051 ± 0.046	1.886 ± 0.038	0.025
Leg Muscles, g	0.977 ± 0.022	0.899 ± 0.020	0.037
BMR, ml O ₂ min ⁻¹	0.97 ± 0.03	1.06 ± 0.02	0.001
PMR, ml O ₂ min ⁻¹	9.99 ± 0.32	8.81 ± 0.26	0.017

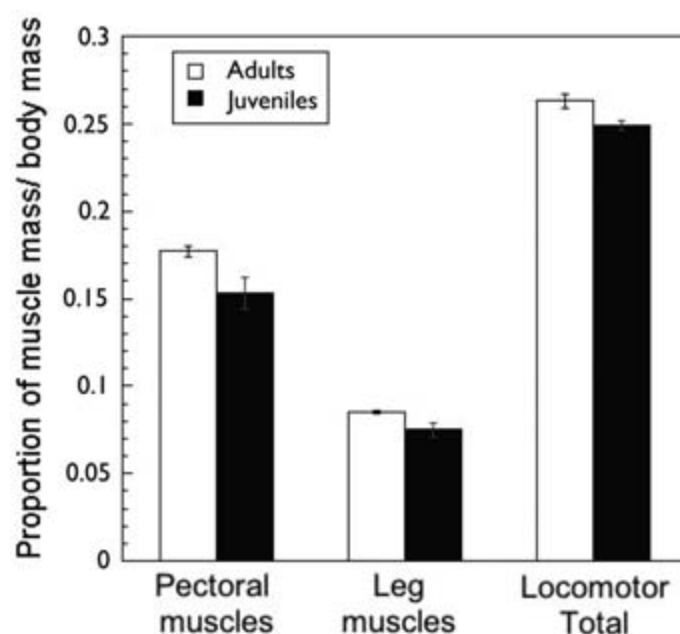


Figure 1. Pectoral, leg, and total (leg + pectoral) muscle masses as proportions of total body mass in adult and juvenile house sparrows. Juvenile proportions are all significantly smaller than those of adults ($P < 0.015$ for all comparisons). Columns represent mean proportions for the 32 adults and 28 juveniles sampled and the error bars designate 1 standard error.

Our measurements of CS activity show that the pectoral muscles of juveniles have significantly higher mass-specific activity, resulting in there being no difference in total muscle CS activity between the age groups (Table 2). The CS levels measured in juvenile pectoral muscles agree closely with values reported by O'Connor and Root (1993) for house sparrows captured in Michigan in late autumn. In contrast to pectoral muscle, leg muscle CS mass-specific activity did not differ between groups, but because leg muscle mass was significantly smaller in juveniles, their total CS activity was significantly lower than in adults (Table 2).

Table 2. Age effects on citrate synthase activity in leg and pectoral muscles of 32 adult and 28 juvenile house sparrows. Statistical comparisons are based on ANCOVA with body mass as a covariate. P values of less than 0.05 identify statistically significant differences between adults and juveniles. Specific activity (SA) is in relation to a gram of muscle tissue, whereas total activity (TA) is the product of specific activity and total mass of a given muscle.

Variable, units	Adults	Juveniles	P Adults vs. Juv.
Leg SA, $\mu\text{mol min}^{-1} \text{g}^{-1}$	67.8 ± 2.3	61.7 ± 2.2	0.088
Leg TA, $\mu\text{mol min}^{-1}$	132.7 ± 5.6	111.4 ± 4.8	0.008
Pectoral SA, $\mu\text{mol min}^{-1} \text{g}$	173.8 ± 4.8	197.8 ± 5.7	0.028
Pectoral TA, $\mu\text{mol min}^{-1}$	707.8 ± 24.7	751.3 ± 30.7	0.091

If characterisation of CS activity in muscles better accounts for their resting metabolic needs as suggested by Vezina and Williams (2005), we would expect the total CS activity, the product of CS mass-specific activity and total muscle mass, to better correlate with BMR than muscle mass alone. After controlling for body mass influences, the correlation between pectoral total CS activity and BMR in both groups combined is, in fact, lower ($r = 0.495$) than for pectoral muscle mass alone ($r = 0.506$; 47 df). Furthermore, total pectoral CS of juvenile sparrows showed much weaker correlation with BMR ($r = 0.39$; $P = 0.043$) than did wet pectoral mass ($r = 0.56$; $p = 0.003$). This trend is also evident for leg muscles in that the correlation between leg muscle total CS activity and BMR was weaker ($P = 0.51$) than for leg muscle mass alone ($P = 0.18$). On the other hand, if CS activity is a valid indicator of tissue oxidative capacity (Emmett & Hochachka 1981; Hochachka et al. 1988), we would expect total pectoral CS activity to have a stronger correlation with PMR than pectoral mass alone. Somewhat surprisingly after controlling for body mass, variation in pectoral muscle mass better explains PMR differences among all sparrows measured ($r = 0.58$, $P < 0.001$; Fig.

2) than does variation in pectoral total CS activity ($r = 0.29$, $P = 0.03$; Fig. 3). Among adult sparrows, PMR was significantly correlated with pectoral mass ($r = 0.63$, $p = 0.001$), but not with pectoral total CS activity ($r = 0.33$, $P = 0.07$). In contrast, both pectoral mass and pectoral total CS activity were significantly correlated with PMR in juveniles ($P < 0.035$ for both comparisons).

Assuming pectoral total CS activity to be a valid descriptor of that muscle's aerobic capacity, we would have to conclude that the significantly lower PMR of juveniles is not due to age-related differences in pectoral muscle oxidative potential. This assumption depends, of course, on how well muscle CS activity describes its aerobic capability. It is revealing that pectoral muscle mass-specific CS activities tend to be slightly higher in chicks about to fledge than in adults in both common terns (*Sterna hirundo*; Burness et al. 2005) and barnacle geese (*Branta leucopsis*; Bishop et al. 1995). Given the absence of flight experience in these chicks, it would be very surprising if their flight PMR could match that of experienced adults. On the other hand, Hammond et al. (2000) found that male red junglefowl (*Gallus gallus*) had significantly higher PMR and muscle citrate synthase activities than females,

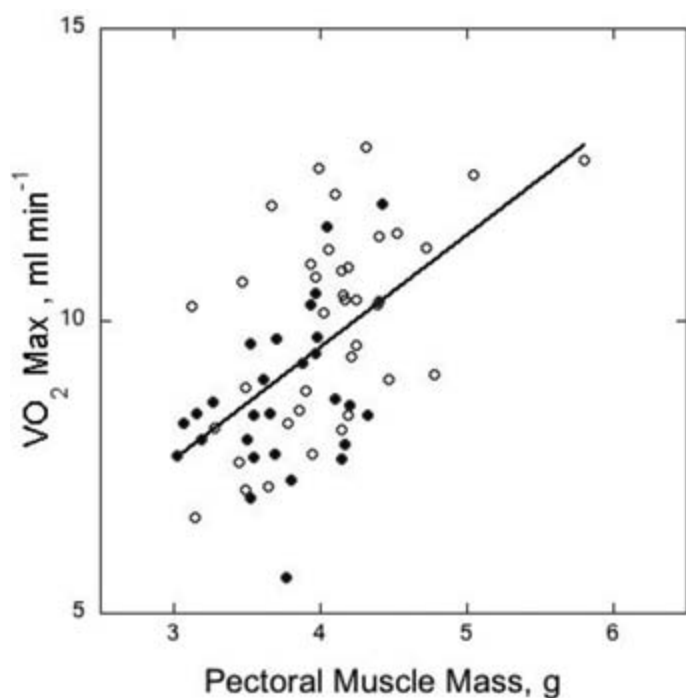


Figure 2. Peak metabolic rate during flight exercise in relation to pectoral mass of adult (open circles) and juvenile (filled circles).

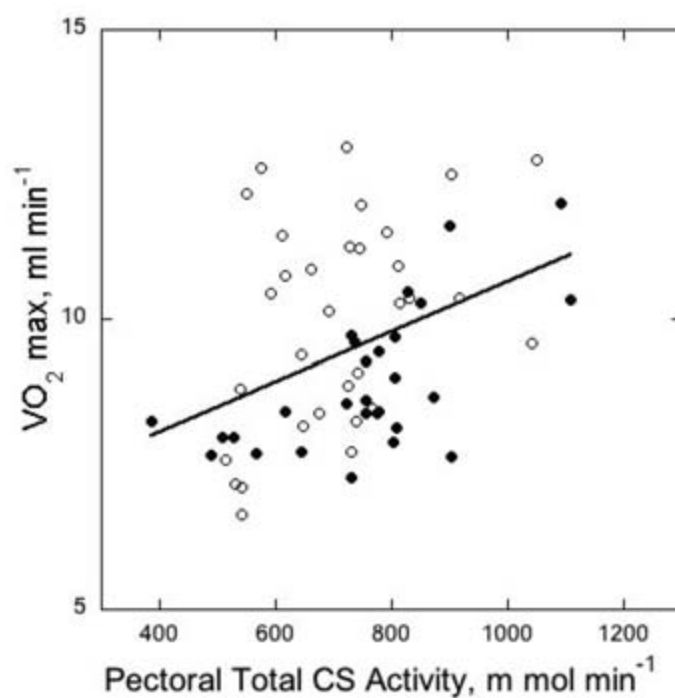


Figure 3. Peak metabolic rate during flight exercise in relation to total citrate synthase activity of adult (open circles) and juvenile (filled circles) pectoral muscles.

but the highly dimorphic nature of this species makes it difficult to gauge the contribution of CS activity to PMR differences. While they did report dominant males to have higher mass-specific CS activities than subordinates, these social groups did not differ in PMR (Hammond et al. 2000). It is also important to recognise that aerobic metabolic pathways involve many enzymes, which rarely

show concordant scaling with overall aerobic performance (Darveau et al. 2005). What we can conclude is that muscle CS activity does not constrain aerobic capacity in juvenile house sparrows. Instead, attainment of adult-level PMR may require ontogenetic changes in morphological structures that are associated with adult peak aerobic performance.

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